9

What is Claimed is:

	1. A genetically modified astrocyte for gene
1	1. A genetically modified astrocyte 101 years
2	therapy, said genetically modified astrocyte
3	comprising:
4	one or more DNA sequences selected from the
5	group consisting of DNA encoding a selectable marker
6	DNA encoding a poison pill, and DNA encoding a
7	molecule useful for gene therapy; and
8	suitable regulatory elements for controlling

expression of said one or more DNA sequences.

- The genetically modified astrocyte of claim 2. 1 1 wherein said selectable marker/comprises neomycin 2 resistance. 3
- The genetically modified astrocyte of claim 1 1 wherein said selectable marker comprises 2 methotrexate resistance. 3
- The genetically modified astrocyte of claim 1 1 wherein said poison pill comprises herpes virus 2 thymidine kinase. 3
- The genetically modified astrocyte of claim 5. 1 1 wherein expression of said DNA encoding said 2 molecule useful for gene therapy results in the 3 production of a protein.
- The genetically modified astrocyte of claim 1 1 wherein expression of said DNA encoding said 2 molecule useful for gene therapy results in the 3 production of anti-sense RNA.

- 7. The genetically modified astrocyte of claim
 wherein expression of said DNA encoding said
 molecule useful for gene therapy results in the
 production of a ribozyme.
- 1 8. The genetically modified astrocyte of claim 2 5 wherein said protein comprises a growth factor.
- 9. The genetically modified astrocyte of claim
 8 wherein said growth factor comprises a cytokine.
- 1 10. The genetically modified astrocyte of claim
 2 5 wherein said protein comprises tyrosine
 3 hydroxylase.
- 1 11. The genetically modified astrocyte of claim
 2 1 wherein said suitable regulatory elements include a
 3 regulatable promoter.
- 1 12. The genetically modified astrocyte of claim
 2 11 wherein said regulatable promoter comprises an
 3 inducible promoter.
- 1 13. The genetically modified astrocyte of claim 2 12 wherein said inducible promoter comprises a human 3 preproenkephalin promoter.
- 1 14. The genetically modified astrocyte of claim 2 11 wherein said regulatable promoter comprises a 3 constitutive promoter.
- 1 15. The genetically modified astrocyte of claim 2 1 wherein said suitable regulatory elements include 3 an astrocyte-specific promoter.

. •

2

3

4

1

2

3

4

5

6

7

1 16. The genetically modified astrocyte of claim 2 15 wherein said astrocyte-specific promoter comprises 3 a promoter for glial fibrillary acidic protein.

- 1 17. An astrocyte cell line comprising the genetically modified astrocyte of claim 1.
- 18. A plasmid for transfection of astrocytes

 which plasmid comprises DNA encoding a molecule

 useful for gene therapy and suitable regulatory

 elements for controlling expression of said molecule

 useful for gene therapy.
- 19. A plasmid for transfection of astrocytes which plasmid comprises DNA encoding a selectable marker and suitable regulatory elements for controlling expression of said selectable marker.
 - 20. The plasmid of claim 19 further comprising DNA encoding a poison pill and further suitable regulatory elements for controlling expression of said poison pill.
- 21. A plasmid for transfection of astrocytes which plasmid comprises DNA encoding a poison pill and suitable regulatory elements for controlling expression of said poison pill.
 - 22. An astrocyte stably transfected with one or more plasmids said one or more plasmids selected from the group consisting of:
 - a plasmid comprising DNA encoding a molecule useful for gene therapy and suitable regulatory elements for controlling expression of said molecule useful for gene therapy;

marker and suitable regulatory elements for controlling expression of said selectable marker; a plasmid comprising DNA encoding a selectable marker and suitable regulatory elements for controlling expression of said selectable marker, and further comprising DNA encoding a poison pill and further suitable regulatory elements for controlling expression of said poison pill; and a plasmid comprising DNA encoding a poison pill and a plasmid comprising DNA encoding a poison pill and suitable regulatory elements for controlling
expression, of said poison pill.

- 23. A method of stably transfecting primary cells, said method comprising stably transfecting said primary cells using non-viral transfection methods.
- 24. The method of claim 23 wherein said nonviral transfection method comprises chemical transfection.
- 1 25. The method of claim 24 wherein said 2 chemical transfection comprises stable calcium 3 phosphate transfection.
- 26. The method of claim 23 wherein said non viral transfections method comprises electroporation.
- 1 27. The method of claim 23 wherein said primary cells comprise astrocytes.
- 1. 28 A method for gene therapy in the central nervous system of a subject which method comprises:

4

5

6

7 8

9

10

11

1

2

3





genetically modifying primary cells to include DNA encoding a molecule useful for gene therapy in the central nervous system; transplanting said genetically modified primary cells into the central nervous system of a subject; and expressing said DNA encoding said moleçule,

thereby producing said molecule for gene therapy in the central nervous system of the subject.

- The method of claim 28 wherein said primary 1 cells comprise astrocytes. 2
- The method of claim 29 wherein said 1 astrocytes are genetically modified by a non-viral 2 transfection method. 3
- The method of claim 30 wherein said non-1 viral transfection method/comprises chemical transfection. 3
 - The method of claim 31 wherein said chemical transfection comprises stable calcium phosphate transfection.
- The method of claim 28 wherein said 1 expression of said DNA is controlled by a regulatable 2 3 promoter.
- The method of claim 33 wherein said 1 regulatable promoter is controlled pharmacologically. 2
- The method of claim 34 wherein said 1 pharmacologic control comprises utilizing 2 dopaminergic pathways. 3

The method of claim 33 wherein said regulatable promoter comprises an inducible promoter. 1 2 The method of claim 33 wherein said, 37. 1 regulatable promoter comprises a constitutive 2 promoter. 3 A method of maintaining and growing 384 1 astrocytes in culture, said method comprising: growing first astrocytes with a /liquid medium 2 overlying said first astrocytes so as to condition 3 4 said liquid medium; removing said conditioned liquid medium; and 5 placing said removed conditioned liquid medium 6 over second astrocytes, said removed conditioned 7 liquid medium capable of maintaining and growing said 8 9 second astrocytes in culture. 10 A method of selecting for astrocytes in a mixed cell population, said method comprising: 1 stably transfecting a mixed cell population with 2 3 an astrocyte-specific plasmid, said astrocytespecific plasmid comprising DNA encoding a selectable 4 5 marker and suitable regulatory elements for controlling expression of said selectable marker; 6 growing said transfected mixed cell population 7 8 under selective conditions, wherein said astrocyte-9 specific promoter functions only in transfected astrocytes present in said transfected mixed cell 10 11 population, such that only transfected astrocytes present in said transfected mixed cell population can 12 be selected under said selective conditions using 13 14 said selectable marker under control of said 15 astrocyte-specific promoter; and

		in white call
1	17	selecting said astrocytes from said mixed cell
	18	population. /
	1 2 3	40. The method of claim 39 wherein said astrocyte-specific promoter comprises a promoter for glial fibrillary acidic protein.
	1 2	41. The method of claim 39 wherein said selective marker comprises neomycin resistance.
	1 2	42. The method of claim 39 wherein said selective marker comprises methotrexate resistance.
		43. The method of claim 41 wherein said
1	1	include exposing sald
ind.	2	selective conditions include the selective conditions in selective conditions include the selective conditions in selective conditions in selective conditions in selective conditions in selective co
I Him thus then I I was the the	3 4	analogue.
7		44. The method of claim 43 wherein said
	1 2	neomycin analogue comprises G418.
		45. The method of claim 42 wherein said
Ų U	1	include exposing sala
	2	transfected mixed cell population to methotrexate.
=	3	
	_	46. A method of expressing a biologically
	1	in an astrocyte of a subject william
	2	and comprises:
	3	/ a sample of an astrocyce,
	4 5	incerting DNA encoding a biologically
	6	into DNA of said astrocyte,
	7	. furnianting said resulting ascission
		/ .
	9	expressing said biologically active more
	10	// said subject.

4

5

6

7

8

10

11

12

1	47. The method of claim 46 wherein said biologically active molecule is selected from the
2	biologically active morecure is selected to a
3	group consisting of a protein, antisense RNA, and a
4	ribozyme.
1	48. The method of claim 46 wherein said sample
2	of an astrocyte is obtained by removing astrocytes
3	from said subject.
1	49. The method of claim 46 wherein said stable

- insertion comprises a non-viral/transfection method. 2
- The method of claim 46 wherein said 50. 1 expression of said biologically active molecule is 2 under control of a regulatable promoter. 3
- The method of claim 50 wherein said 1 regulatable promoter comprises an inducible promoter. 2
- The method of claim 50 wherein said 1 regulatable promoter comprises a constitutive 2 promoter. 3
 - A method of killing astrocytes in a subject, said method comprising:

2 obtaining a sample of astrocytes; 3

stably transfecting said astrocytes with a plasmid / said plasmid comprising DNA encoding a poison/pill and suitable regulatory elements for controlling expression of said poison pill;

transplanting said transfected astrocytes into a

subject; and 9

exposing said transplanted transfected astrocytes to a selective condition, wherein said suitable regulatory elements cause expression of said

2

3

5

6

7

8

9 10

11

13 14	DNA encoding said poison pill only in said transplanted transfected astrocytes present in said
15	that only said transplanted cransplanted
To	said subject are killed of bell
16	selective condition due to said expression of said
17	selective condition due to salu expression
	DNA encoding said poison pill under control of said
18	DNA encouring but i
19	astrocyte-specific promoter.

- 54. The method of claim 53 wherein said poison
 pill comprises herpse virus thymidine kinase.
- 55. The method of claim 54/wherein said
 exposure to a selective condition comprises exposure
 to a drug selected from the group consisting of
 acyclovir and gancyclovir.
 - 56. A method of preventing deterioration of phenotypically normal cells in a subject which comprises:

detecting a genotype indicative of an eventual phenotypic abnormality in said normal cells;

treating said normal cell with the genetically modified astrocyte of claim 1 so as to prevent said phenotypic abnormality, said prevention being by expression of said DNA encoding said molecule useful for gene therapy by said genetically modified astrocyte.

- 57. The method of claim 56 wherein said
 phenotypic abnormality is indicative of Huntingtons
 disease.
- 1 58. An astrocyte maintained and grown by the 2 method of claim 38.

	,
_	59. An astrocyte selected by the method of
1	claim 39.
2	Claim 33.
	60. A kit for gene therapy comprising the
1	genetically modified astrocyte of claim 1
2	genetically modified doctor
	61. A kit for gene therapy comprising the
1	genetically modified astrocyte of claim 17.
2	genetically modified doctory
	62. A kit for gene therapy comprising one or
1	more plasmids, said one or more plasmids selected
2	more plasmids, said one of more plasmids,
3	from the group consisting of: a plasmid comprising DNA encoding a molecule
4	useful for gene therapy and suitable regulatory
5	useful for gene therapy and surtuals required to said molecule elements for controlling expression of said molecule
6	elements for controlling explanation of
7	useful for gene therapy; a plasmid comprising DNA encoding a selectable
8	a plasmid comprising but encouring
9	marker and suitable regulatory elements for
10	controlling expression of said selectable marker;
11	a plasmid comprising DNA encoding a selectable
12	marker and suitable regulatory elements for
13	controlling expression of said selectable marker, and
14	further comprising DNA encoding a poison pill and
15	further comprising the further suitable regulatory elements for controlling
16	expression of said poison pill; and
17	a plasmid comprising DNA encoding a poison pill
18	and suitable regulatory elements for controlling
19	expression of said poison pill.
1	63. The kit of claim 62 further comprising
2	astrocytes to be transfected with said one or more
3	plasmids.
•	
1	64. A kit for gene therapy comprising:
_	,

9	plasmid vector having a polylinker site for
	tion of DNA encoding a gene of interest;
insert	restriction enzymes for inserting said DNA at
said s	site; and
1	the astrocyte of claim 58 to be transfected by
the p	lasmid vector after insertion of said DNA into
said	plasmid vector./